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OPTIMIZATION OF CELLULASE PRODUCTIVITY

FROM ACIDOTHERMUS CELLULOLYTICUS

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ABSTRACT

The production of cellulase activity by Acidothermus cellulolyticus was studied by using media of various substrate composition. Controlled (pH, temperature and dissolved oxygen) fermentations with 1.0, 0.5 and 0.25 (w/v) percent of cellobiose were analyzed for filter paper activity, reducing sugar and optical density as a functiion of time. Similar analyses of a fermentation with 0.1 (w/v) percent cellobiose and 1.0 (w/v) percent solka floc showed approximately 2X, 2X and 3X as much filter paper degrading activity as the three respective cellobiose fermentations.

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INTRODUCTION

Cellulase enzymes from thermophilic cellulolytic bacteria possess attributes preferred to those enzymes obtained from mesophilic and thermotolerant fungal cellulase producers (Tucker et al., 1987). Acidothermus cellulolyticus is a thermophilic aerobic bacterium which produces cellulolytic enzymes (Mohagheghi et al., 1986). The specific growth rate and cell density acheived by thermophilic aerobic bacteria are potentially greater than those parameters obtained with mycelial cultures or anaerobic thermophilic clostridial cellulase producers (Sissons et al., 1987). Consequently, if the specific productivity of cellulase activity were even equivalent to that of other systems, A. cellulolyticus would economically compete as a source of cellulase enzymes with desirable thermotolerant properties. Hence, the overall objective of the study is to define methods of obtaining high cellulase productivities from fermentations of this organism. Optimization of cell growth and induction of cellulase production will be approached by medium supplementation in controlled fermentations.

EXPERIMENTAL DESCRIPTION

Microorganism

A culture of a cellulolytic thermophilic bacteria, which was isolated from Yellowstone Park basins by SERI investigators (Mohagheghi et al., 1986) and designated as A. cellulolyticus 11-B, was used for these studies.

Medium

The lowphosphate basal salts medium (LPBM) described by Mohagheghi et al. (1986) was used in fermentor runs in 2x concentration with 20 mg/l $FeSO_4$ and 0.5 g/l of yeast extract. Cellobiose concentration was varied as described in the text.

Fermentation Conditions

Fermentors (B. Braun Biostat V) of 2.5 l working volume were used. By addition of 1.0 N NaOH and 1.0 N H_2SO_4, pH was maintained at 5.2. Temperature was maintained at 55°C. Dissolved oxygen was not allowed below 40 percent of saturation by increasing aeration rate at constant agitation (150 rpm).

Inoculum

Inoculum was prepared from 1.0 ml of frozen culture containing DMSO (0.07 ml/ml of culture). A culture, which was stored in this way, was inoculated into 20 ml of medium. Growth at 55°C in an incubator shaker for 24 hours prepared the inoculum for the 2.5 l sterilized fermentor.

Assays

Filter paper assay and reducing sugar determinations were conducted according to the recommendations of the Measurement of Cellulase Activities prepared for the IUPAC (Ghosh, 1987). International units of filter paper degrading activity were reported.

Cell density was measured by absorption at 600 nm, dilution of dense cultures with distilled water was conducted to determine absorption values.

RESULTS

Initial experimental work was begun in SERI Laboratories with shake flask cultures of A. cellulolyticus growing on various cellulosic substrates. Since pH control was obviously necessary, subsequent studies were conducted in 5 liter B. Braun V units at pH 5.2, and 55°C with dissolved oxygen maintained at greater than 40% of saturation. The initial substrate concentration was varied in four batch fermentations.

Cellobiose: 10 g/l

The culture grew fast begining 17 hours after inoculation and continuing at a linear rate through 64 hours (Figure 1). Approximately 60 percent of the cellobiose was utilized during this period. The average concentration of cellulase activity in the fermentation broth was 0.02 IU/ml. High levels of reducing sugar in the medium necessitated 20 fold dilution of the assay mixture, which reduced the accuracy of the filter paper assay.

Cellobiose: 5 g/l

Cell growth in this fermentation stopped at 41 hours after inoculation, which was coincident with depletion of cellobiose from the medium (Figure 2). At the same point in time cellulase production began. An increase of 0.02 IU/ml occured over a six hour period after which production stopped.

Cellobiose: 2.5 g/l

The time course of this fermentation was similar to that containing 0.5 (w/v) percent initial substrate concentration (Figure 3). Cellobiose depletion occured at 22 hours after inoculation and within 4 hours, 0.028 IU/ml of cellulase activity has formed. Protolytic enzymes released from cell lysis resulted in a decline in filter paper degrading activity.

Cellobiose 1g/l + Solka Floc 10 g/l

Measurement of optical density was not possible in this fermentation containing solka floc (Figure 4). Reducing sugar concentration was constant for 48 hours. Enzyme production beginning at 48 hours resulted in a reduction of cellobiose concentration. Linear accumulation of filter paper activity occured for 20 hours before termentation of the experiment.

Figure 1.

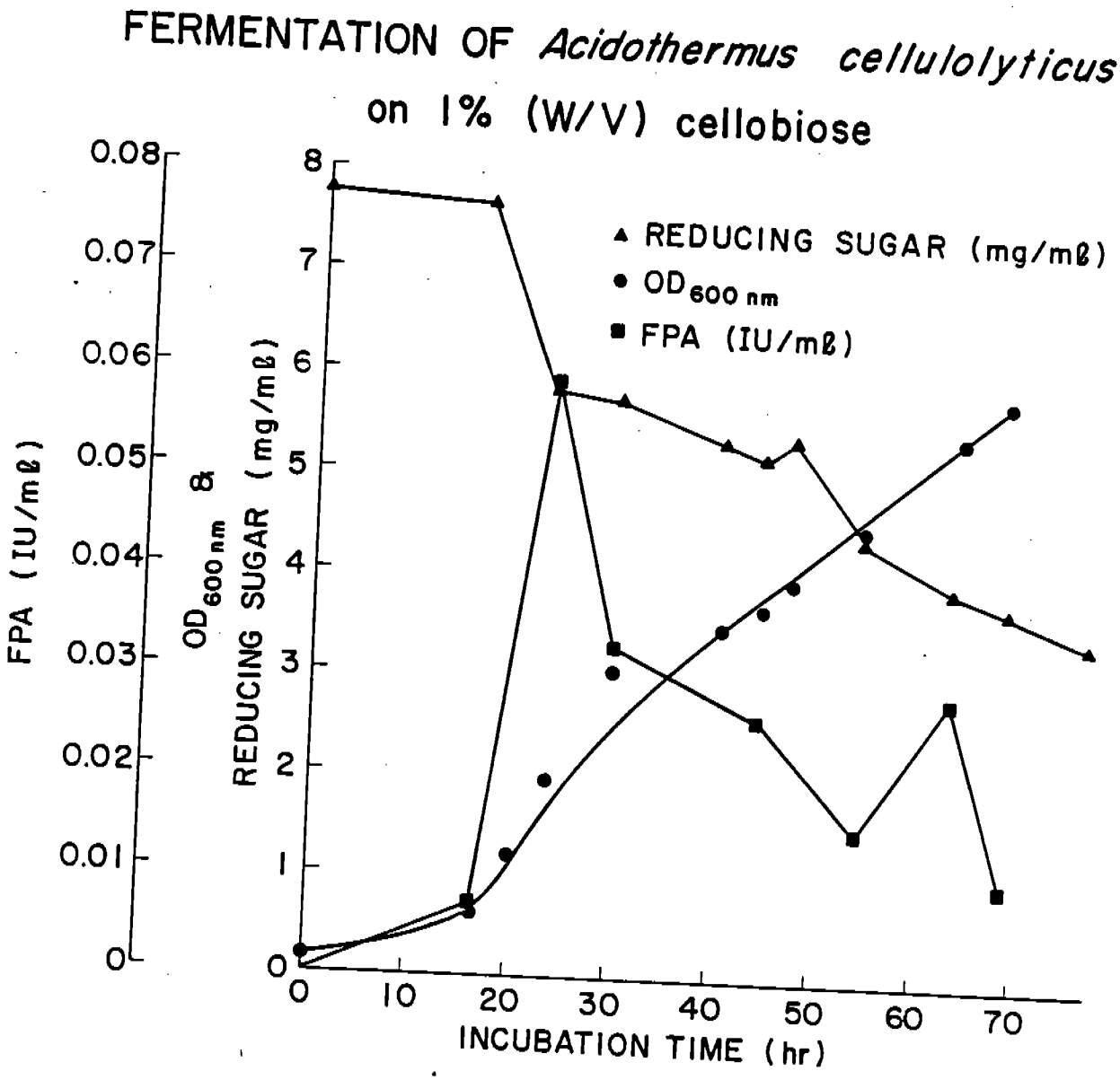

FERMENTATION OF Acidothermus cellulolyticus
on 1% (W/V) cellobiose
FPA (IU/mℓ)
OD600 nm &
REDUCING SUGAR (mg/mℓ)
INCUBATION TIME (hr)
▲ REDUCING SUGAR (mg/mℓ)
● OD600 nm
■ FPA (IU/mℓ)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
8
7
6
5
4
3
2
1
0
0
10
20
30
40
50
60
70

Figure 2

FERMENTATION OF *Acidothermus cellulolyticus* on 0.5% (W/V) cellobiose

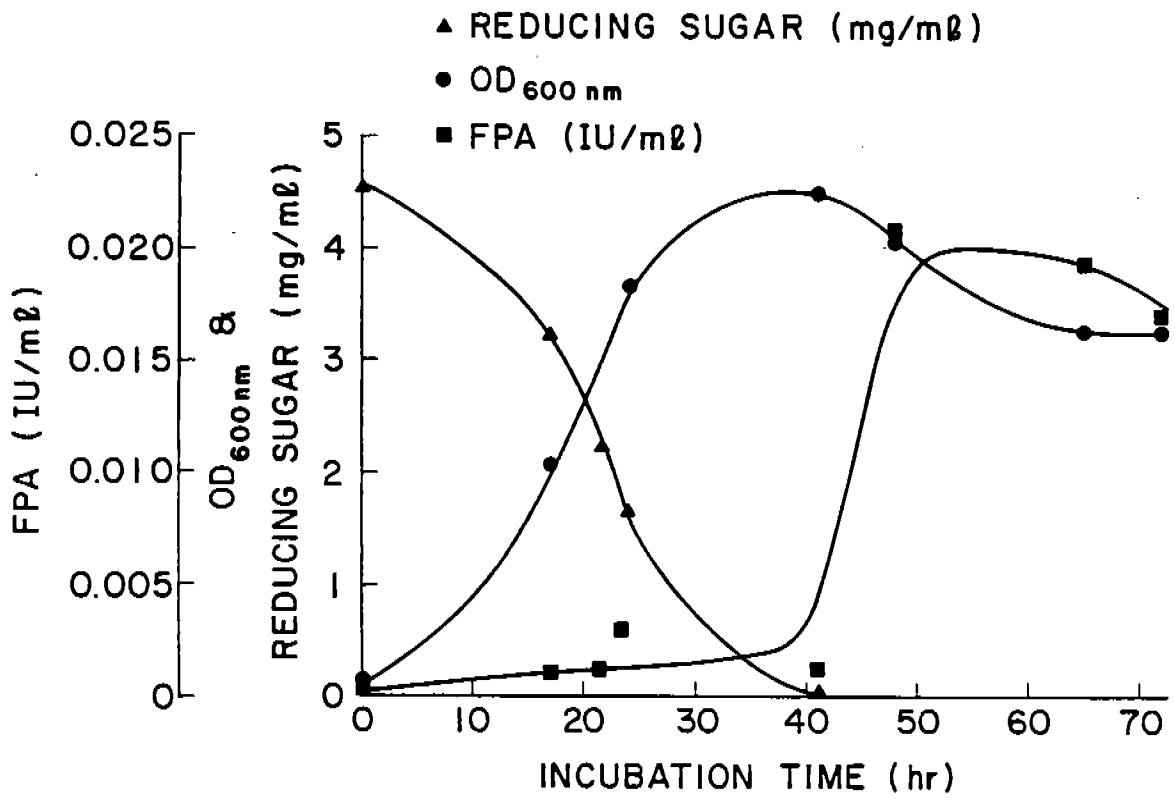


Figure 3

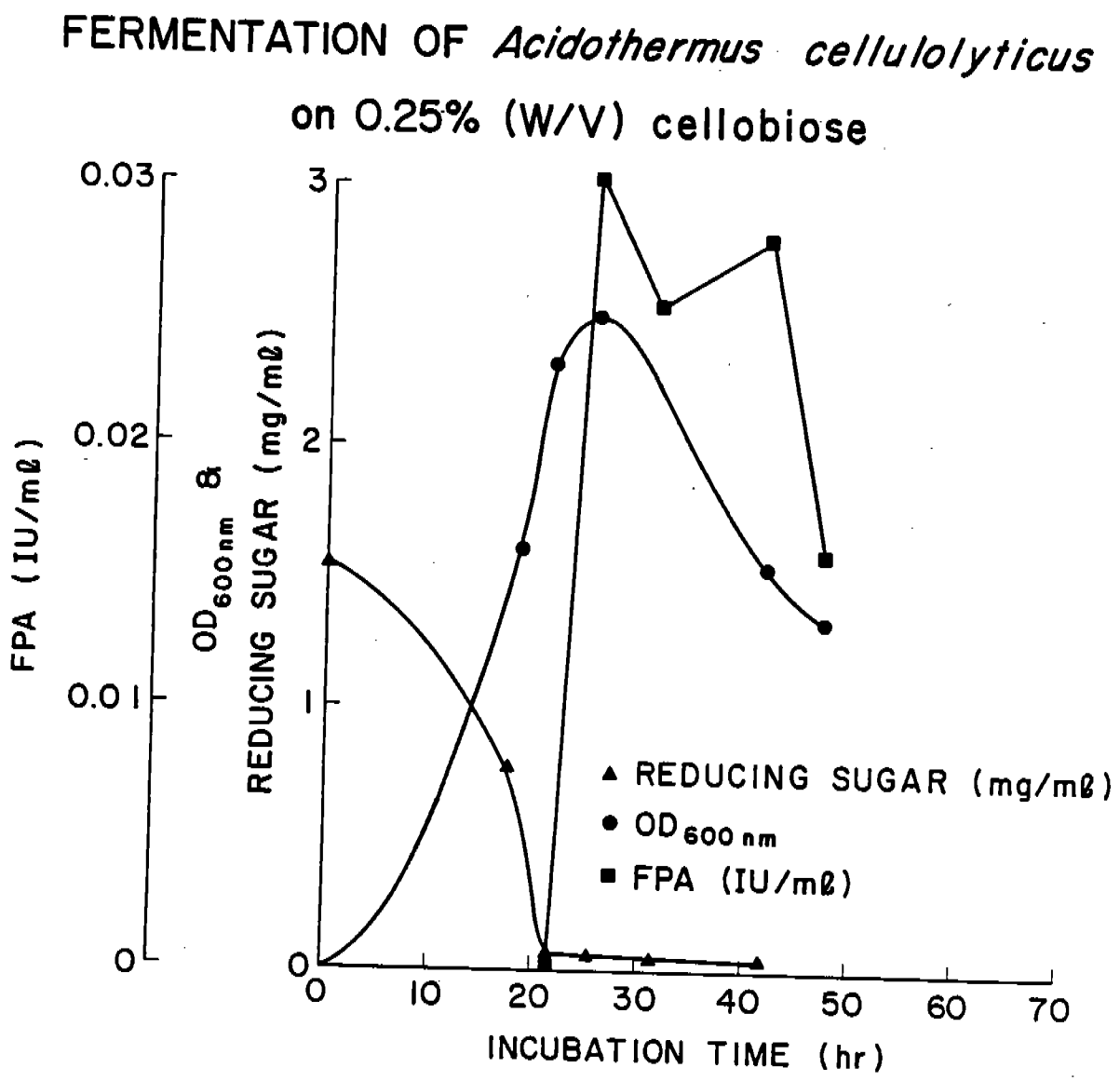
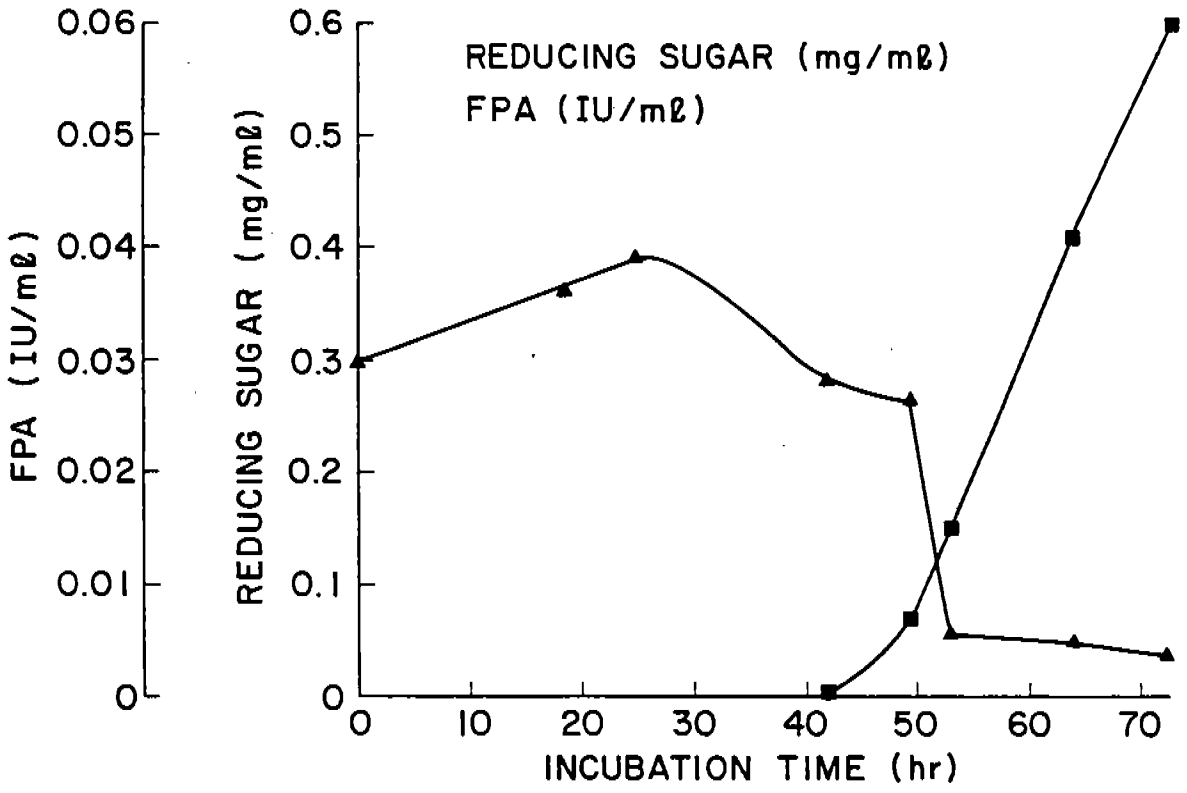

FERMENTATION OF *Acidothermus cellulolyticus*
on 0.25% (W/V) cellobiose
FPA (IU/mℓ)
0.03
0.02
0.01
0
OD600nm &
REDUCING SUGAR (mg/mℓ)
3
2
1
0
▲ REDUCING SUGAR (mg/mℓ)
● OD600 nm
■ FPA (IU/mℓ)
0
10
20
30
40
50
60
70
INCUBATION TIME (hr)

Figure 4

FERMENTATION OF *Acidothermus cellulolyticus* on 0.1% cellobiose with 0.5% solka floc



DISCUSSION

The following fermentation parameters were estimated from data obtained during the course of fermentations.

Initial Substrate Conc.	Growth			Enzyme Productivity		
	Lag Phase (hr)	Max.O.D. (A600)	u (hr)	Lag Phase (hr)	Max.Act. (U/ml)	Product. (U/l.hr)
1% CB	17	3.0	0.09	--	--	--
0.5% CB	17	3.6	0.08	41	0.02	2.80
0.25% CB	17	2.3	0.06	21	0.03	7.50
0.1% CB + 0.5% Solka Floc	--	--	--	50	0.04	2.3

*CB: cellobiase

These data and fermentation time course curves indicate the following:

1. Lag phase for growth was constant.
2. Maximun specific growth rate was dependent on initial substrate concentration.
3. Enzyme productivity was inversely dependent on initial substrate concentration.
4. Enzyme synthesis was delayed by addition of cellulose to initial substrate charge.
5. Addition of cellulose to initial substrate charge diminished enzyme productivity.
6. Sustained culture viability was dependent on carbonsource nutrient supply.

FUTURE WORK

The overall objective of the proposal, to improve the volumetric productivities of cellulase enzymes from A. cellulolyticus, will be approached through

1. determination of optimum conditions for high cell density production,
2. investigation of nutritional and/or metabolic controls important to expression of cellulase enzymes, and
3. selection of mutant strains for hypercellulase production or secretion.

Based on results from the fermentation in which 1 g/l cellobiose and 10 g/l solka floc were used, reduced lag time in cell growth would expected if repeated using 2.5 g/l of cellobiose and 10 g/l solka floc. Fedbatch fermentation would be started with an initial cellobiose concentration of 2.5 g/l. Systematically planned experimentation, in which constant cellobiose levels would be maintained, will be based on substrate utilization rate in controlled fermentations at specific set points of 0.1 g/l, 0.2 g/l, 0.3 g/l, 0.4 g/l, and 0.5 g/l.

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